IN VIVO ANTIHYPERLIPIDEMIC ACTIVITY OF ETHANOLIC FRUIT EXTRACT OF *Solanum virginianum* LINN.,

AND

IN SILICO PROFILING OF SOME OF ITS ISOLATED CONSTITUENTS

A Dissertation submitted to

THE TAMILNADU DR. M.G.R. MEDICAL UNIVERSITY

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in partial fulfilment of the requirements for the award of the degree of

MASTER OF PHARMACY IN

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Submitted by

Reg.No. 261426063

Under the guidance of

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CERTIFICATE

This is to certify that this dissertation work entitled ***"IN VIVO* ANTIHYPERLIPIDEMIC ACTIVITY OF ETHANOLIC FRUIT EXTRACT OF *Solanum virginianum* LINN., AND *IN SILICO* PROFILING OF SOME OF ITS ISOLATED CONSTITUENTS"** submitted by **Reg.No 261426063** in partial fulfilment of the requirements for the award of the degree in **MASTER OF PHARMACY IN PHARMACOLOGY** by the Tamilnadu Dr. M.G.R. Medical University, Chennai is a bonafide record of the work done by her in the Institute of Pharmacology, Madras Medical College, Chennai during the academic year 2015-2016 under the guidance of **Dr. N. JAYSHREE, M.Pharm., Ph.D.,** Professor of Pharmacology, Institute of Pharmacology, Madras Medical College, Chennai-600003.

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CONTENTS

LIST OF ABBREVIATIONS USED

ACAT	Acyl Coenzyme A Cholesterol Acetyltransferase
BHT	Butylated Hydroxy Toluene
BW	Body Weight
CH	Cholesterol
CVD	Cardio Vascular Disease
Da	Dalton
DPPH	2, 2- diphenyl -1- picryl hydrazyl radical
GLIDE	Grid based Ligand Docking with Energetics
GPCR	G-protein Coupled Receptor
GSH	Glutathione
HDL-C	High Density Lipoprotein Cholesterol
HMG-CoA	3-Hydroxyl-3-methylglutaryl coenzyme A
ICMR	Indian Council of Medical Research
IDL-C	Intermediate Density Lipoprotein Cholesterol
LDH	Lactate Dehydrogenase
LDL-C	Low Density Lipoprotein Cholesterol
log P	Partition Coefficient
log D	Diffusion coefficient
LPL	Lipoprotein Lipase
MTP	Microsomal triglyceride Transfer Protein
NMR	Nuclear Magnetic Resonance
PDB	Protein Data Bank
PSA	Polar surface area

QSAR	Quantitative Structure Activity Relationship
STZ	Streptozocin
TG	Triglyceride
VLDL-C	Very Low Density Lipoprotein Cholesterol
vHTS	Virtual High Throughput Screening
WHO	World Health Organisation
xp	Extra Precision

INTRODUCTION

Modern culture, in the name of westernization, has changed our life style which in turn has changed our food habits and health care. This has lead to an increased incidence of various disorders like diabetes, hypertension and hyperlipidemia.

Hyperlipidemia is a heterogenous group of disorders characterized by abnormal elevated levels of any or all lipids and/ or lipoproteins in the blood. It is also known as hyperlipoproteinemea and is considered as a key risk factor for cardio vascular disorders (CVD)[1].

The causes of hyperlipidemia are mainly life style changes (poor diet, smoking, alcohol). The hyperlipidemia may be primary ie. Genetic (monogenic, polygenic) or secondary which is associated with diabetes, myxedema, nephrotic syndrome, chronic alcoholism, drugs (corticosteroids, oral contraceptives, β-blockers) etc[2].

At least 3/4th of India's population has abnormal levels of cholesterol that increases the risk of cardio vascular diseases according to a study commissioned by the Indian Council of Medical Research (ICMR). Studies have shown that Indians are affected by heart diseases at a much younger age when compared to the people in the West. According to the statistics provided by the Tamilnadu government, 1/4th of all deaths among people in the 25-69 years age groups is due to cardio vascular diseases. There have been data on risk factors such as obesity, diabetes, hypertension and lifestyle habits such as poor diet, smoking and alcohol.

A study conducted across 2042 people in Tamilnadu and other cities showed that 4/5th (79%) of population in urban and rural areas had at least one abnormality in lipid parameters. One in ten persons (13%) had high cholesterol level and more than one in five (29.5%) had high levels of triglycerides. To make it worse, 72.3% had low levels of HDL (good cholesterol), 11.8% had high levels of LDL (bad cholesterol). HDL or good cholesterol is universally low across the country. The study group found hypercholestremia in 18.3% of Tamilnadu population[3].

The common treatment for hyperlipidemia is prescription of statins, bile acid sequestrants, fibric acid derivatives and nicotinic acid. Adverse effects associated with these drugs are headache, nausea, bowel upset, rashes, sleep disturbance, abnormal liver function, myositis, hyperuricemia, rise in serum transaminase, muscle tenderness and rise in Creatine Phosphokinase levels[2].

Traditional treatment

Indigenous systems of medicine like Siddha, Ayurveda and Unani mainly use medicinal plants for treatment of various ailments of human beings and animals. With the development of these systems, herbal plants are being sought after, both by clinicians and patients in search for new cure of diseases. Herbal medicine is a form of complementary and alternative medicine and is becoming increasingly popular in both developing and developed countries[4].

WHO has described traditional medicine as one of the surest means to achieve total health care coverage of the world's population. In pursuance of its goal of providing accessible and culturally acceptable health care for the global population, WHO has encouraged the rational use of traditional plant based medicines by member

states and has developed technical guidelines for the assessment of herbal medicines[5,6].

Herbal drugs have been used throughout the world and have raised greater attention in recent times because of their diverse nature of curing diseases, safety and high levels of tolerance compared to the conventional medicines. Moreover the herbs with natural combinations of constituents as a whole, are naturally occurring remedies which have proved to be more effective and safer than conventional medicines[7].

Herbs that are used as anti hyperlipidemic agents are *Azima tetracantha*, *Cinnamomum tamala*, *Commiphora mukul*, *Curcuma longa*, *Gymnema sylvestre*, *Moringa olifera*, *Prunus persica*, *Sapindus emarginatus*, *Solanum melongena*, *Terminalia arjuna*, *Terminalia palida*, *Terminalia paniculata*[8].

Solanum virginianum Linn., (commonly known as *Solanum xanthocarpum*) belonging to the Solanaceae family, well known as kantangatiri in Tamil, is an annual herbaceous plant mainly growing in India. It is used as a traditional healer of many ailments and has its own importance in Ayurveda to treat fever, cough, asthma, lumbago, piles, urinary diseases, heart diseases and for reducing of fat[9]. It is reported to be non-toxic and safe for human use[10]. All parts of the plant have been found to be noteworthy.

The fruits are used as antidiabetic and anthelmintic, the root is used as an expectorant and is useful in asthma, cough and helps to maintain body temperature. The entire plant is used to treat throat infection and other inflammatory problems.

The present work proposes to evaluate the antihyperlipidemic activity of the fruits of *Solanum virginianum* Linn., after a thorough literature review.

The process of drug discovery is very complex and requires interdisciplinary efforts to design effective and commercially feasible drugs. Earlier, drug discovery was a trial and error process. The process of drug development has evolved with time. New understanding of the quantitative relationship between structure and biological activity ushered the beginning of computer-aided drug design. With the help of computers, a new era has begun in drug discovery. The development cost and time is expected to be cut by almost a third by the use of Computer Aided Drug Design[11].

Isolation of active constituents and synthesizing it to target the receptors is a tedious process. The alternative is to make it possible using *in-silico* studies. There are various softwares like Dock, Auto dock, Argus lab, Glide, Gold, Maestro etc. and various supporting softwares like Chemdraw, Chemsketch, Python, Molgrow etc. available which aid the drug discovery process and make it less tedious[12].

Docking is a search database of molecular structures and retrieves all molecules that can interact with the molecule of interest. It attempts to find the best matching between two molecules. Docking is important to find inhibitors for specific target proteins and to design new drugs. It is acquiring importance as the number of protein structure increases and the efficiency increases accordingly.

Some of the successful outcomes of docking studies are the discovery of Amprenavir (Agenerase) for HIV protease inhibition by GSK and Vertex, Nelfinavir (Viracept) for HIV by Pfizer and Zanamivir (Relenza) for influenza neuraminidase inhibitor by GSK[13].

This study also attempts to evaluate *in silico* antihyperlipidemic activity, mechanism of action, ADME properties and toxicity profiles for some of the already isolated selected compounds of *Solanum virginianum* Linn., after establishing the antihyperlipidemic activity of *Solanum virginianum* Linn., fruits on rats.

HYPERLIPIDEMIA

Dyslipidemia refers to the alteration of one or many of the lipoproteins which may be an elevation of triglycerides or low density lipoprotein cholesterol, or decrease in high-density lipoprotein cholesterol. Elevation of lipid levels alone is termed as Hyperlipidemia[14].

Hyperlipidemia- causes[15]

- Environmental factors
- Genetic factors
- Secondary causes

Environmental factors

Dietary factors and obesity.

Genetic factors

Occur due to single gene or multiple gene defects.

Secondary causes[16]

- Diabetes mellitus
- Hypothyroidism
- Lipodystrophy
- Alcoholism
- Use of anti-hypertensive drugs, diuretics, Glucocorticoid, Protease inhibitor
- Obstructive liver disease
- Nephritic syndrome
- Acute intermittent porphyria

Pathophysiology of hyperlipidemia[17, 18]

Exogenous pathway of lipids

Fat-soluble vitamins, dietary cholesterol and fatty acids are absorbed in the proximal part of the small intestine. Inside the intestinal lumen, the diet TG are hydrolysed by lipases and are also emulsified with bile acids to form micelles.

In the enterocyte, by the addition of a free fatty acid, the cholesterol esterification occurs which results in the formation of cholesteryl esters. Incorporation of triglycerides with fatty acids containing more than 12 carbons atoms are packed with apo-B48, cholesteryl esters, retinyl esters, phospholipids and cholesterol resulting in the formation of chylomicrons.

The newly secreted chylomicrons are called nascent chylomicrons which are absorbed into the intestinal lymph and carried directly through the thoracic duct to the blood stream. They are transported to the peripheral tissues before entering the liver.

In heart, skeletal muscle and adipose tissue, these nascent chylomicrons are attached to the lipoprotein lipase anchored by a protein called phosphatidyl inositol-anchored protein, GPIHBP1. These reactions occur mainly on the endothelial surface of the capillaries. They are hydrolysed by the lipoprotein lipase and the free fatty acids are released. HDL transfers the apo C-II to the chylomicron that acts as a cofactor for lipoprotein lipase.

The released free fatty acids are taken up by heart and skeletal muscles which are oxidized to generate energy. They can also be re-esterified and stored as triglyceride. Some of the free fatty acids released will enter into the hepatocytes by binding with the plasma protein like albumin.

Due to hydrolysation of its hydrophobic core the resultant chylomicrons progressively decrease in size. The hydrophilic lipids like cholesterol, phospholipids

and the protein moiety apolipoproteins on the particle surface are transferred to HDL. These result in the formation of a chylomicron remnant which is about half the diameter of nascent chylomicron.

The chylomicron remnants are mainly made up of cholesterol and cholesteryl esters. These remnants are rapidly taken up by the liver from the circulation where apo-E act as a ligand.

Endogenous pathway of lipids- hepatic lipids

The endogenous transport of cholesterol mainly involves

- The liver which secretes apo-B lipoproteins
- The peripheral tissues where the triglycerides particles are metabolized.

The VLDL particles resemble chylomicrons in protein composition, where the apo-B48 is replaced by apoB-100. They have the higher ratio of cholesterol and triglycerides.

The triglycerides present in the very low density lipoprotein are derived mainly from the esterification of long-chain fatty acids in the liver. The process of combining the hepatic triglycerides with the other major components of the nascent VLDL particle like apoB-100, phospholipids and cholesteryl esters are acquired by the action of the enzyme protein called microsomal triglyceride transfer protein (MTP).

In the plasma, HDL transfers the apo-E and the C series of apolipoproteins to the VLDL particle. In the heart, skeletal muscle and adipose tissue, the triglycerides of the VLDL particle are hydrolysed by the lipoprotein lipase enzyme, a process similar to the one occurring to the chylomicron. This results in the formation of VLDL remnants which are called as IDL (intermediate density lipoprotein).

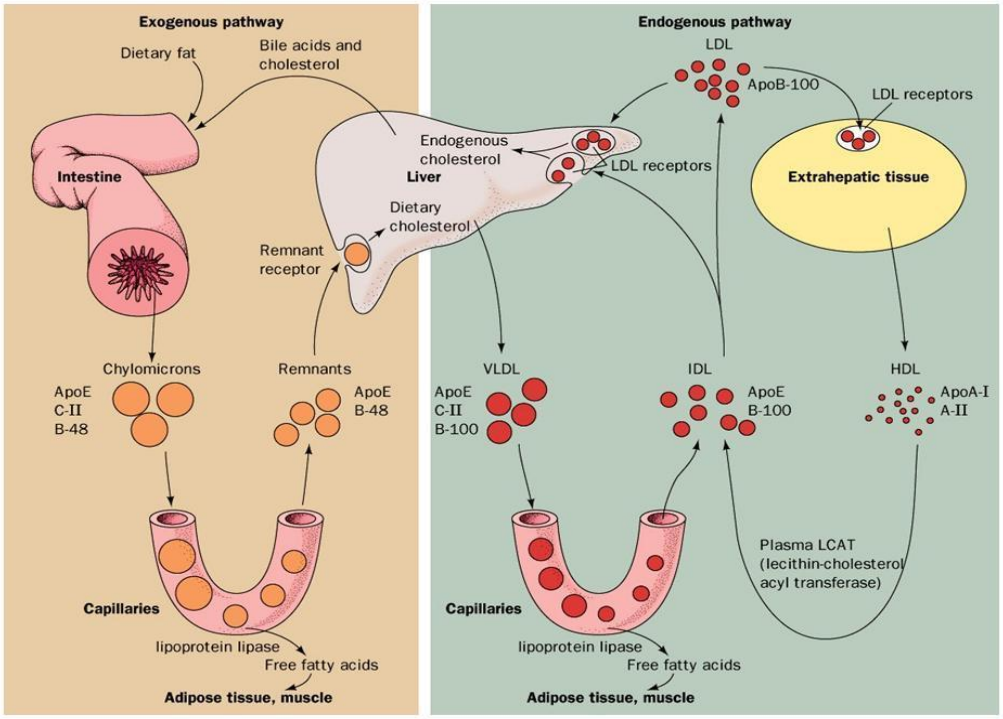


Figure 1: Exogenous and Endogenous pathway of lipids

In the plasma, HDL transfers the apo-E and the C series of apolipoproteins to the VLDL particle. In the heart, skeletal muscle and adipose tissue, the triglycerides of the VLDL particle are hydrolysed by the lipoprotein lipase enzyme, a process similar to the one occurring to the chylomicron. This results in the formation of VLDL remnants which are called as IDL (intermediate density lipoprotein).

IDL contains almost same amounts of triglyceride and cholesterol. 40-60% of IDL particle are removed by the liver through endocytosis by binding to apo-E and apoB-100. The remaining IDL is remodeled by hepatic lipase enzyme to form LDL.

In this process most of the triglycerides are hydrolysed and result in the formation of LDL which carries apoB-100. In most of the individuals, the concentration of plasma cholesterol is equivalent to the amount of cholesterol present in the LDL particle. In the liver, about 70% of circulating LDL cholesterol is cleared by LDL receptor-mediated endocytosis.

Table 1: Classification of plasma lipid levels[19]

Type of Cholesterol	Values
LDL Cholesterol	• <100 mg/dL Optimal • 100-129 mg/dL Near/above optimal • 130-159 mg/dL Borderline high • 160-189 mg/dL High • >190 mg/dL Very high
Total Cholesterol	• <200 mg/dL Desirable • 200-239 mg/dL Borderline high • > 240 mg/dL High
HDL Cholesterol	• < 40 mg/dL Low • 40-60 mg/dL Normal • >60 mg/dL High
Triglycerides	• <150 mg/dL Normal • 150-199 mg/dL Borderline high • 200-499 mg/dL High • >500 mg/dL Very high

Table 2: Drugs commonly used in the treatment of hyperlipidemia[20, 21]

Drug (daily dose)	Mechanism of action	Effect on lipids (%)
1. <u>HMG-CoA reductase inhibitors</u> • Lovastatin (10-80 mg) • Simvastatin (5-40 mg) • Atorvastatin (10-80 mg) • Rosuvastatin (5-20 mg)	↓Cholesterol synthesis by inhibition of rate limiting HMG-CoA reductase.	LDL ↓ 20-55 HDL ↑ 15-30 TG ↓ 10-35
2. <u>Bile acid sequestrants</u> • Cholestyramine (4-16 g) • Colestipol (5-30 g)	↓ Bile acid absorption, ↑ hepatic conversion of CH to bile acids, ↑ LDL receptors on hepatocytes.	LDL ↓ 15-30 HDL ↑ 3-5 TG not affected
3. <u>Fibric acid derivatives</u> • Gemfibrozil (1200 mg) • Bezafibrate (600 mg) • Fenofibrate (200 mg)	↑ Activity of lipoprotein lipase, ↓ release of fatty acids from adipose tissue	LDL ↓ 20-55 May ↑ LDL when TG is high HDL ↑ 15-30 TG ↓ 10-35
4. Nicotinic acid (2-6 g)	↓ Production of VLDL, ↑Lipolysis in adipocytes.	LDL ↓ 15-25 HDL ↑ 20-35 TG ↓ 20-50

COMPUTER AIDED DRUG DESIGN

Computer aided drug design uses computational chemistry to discover, enhance or study drugs and related biologically active molecules. The most fundamental goal is to predict whether a given molecule will bind to a target and if so, how strong the binding would be. Molecular mechanics or molecular dynamics are most often used to predict the conformation of the small molecule and to model conformational changes in the biological target that may occur when the small molecule binds to it. This provides semi-quantitative prediction of the binding affinity. Also, knowledge-based scoring function may be used to provide binding affinity estimates. These methods use linear regression, machine learning, neural nets or other statistical techniques to derive predictive binding affinity equations by fitting experimental affinities to computationally derived interaction energies between the small molecule and the target[22].

Rational drug design[23]

Rational drug design is the strategy of creating new molecules with a certain functionality, based upon the ability to predict how the structure of the molecule will affect its behavior through physical models. This can be done either from scratch or by making calculated variations on a known structure and is usually contrasted with direct evolution. Rational drug designing is a method of finding new medications, based on the biological receptors and target molecules. The objective of drug design is to find a chemical compound that can fit to a specific cavity on a protein target both geometrically and chemically.

Types of drug design

- Ligand based drug design
- Structure based drug design

Ligand based drug design

Ligand based drug design is an indirect approach which relies on knowledge of other molecules that bind to the biological target of interest. These other molecules may be used to derive a pharmacophore model that defines the minimum necessary structural characteristics a molecule must possess in order to bind to the target. In other words, a model of the biological target may be built based on the knowledge of what binds to it and this model in turn may be used to design new molecular entities that interact with the target.

Structure based drug design[24]

Structure based drug design is a direct approach which relies on knowledge of the three dimensional structure of the biological target obtained through methods such as x-ray crystallography and NMR spectroscopy. If an experimental structure of a target is not available, it may be possible to create a homology model of the target based on the experimental structure of a related protein. Using the structure of the biological target, candidate drugs that are predicted to bind with high affinity and selectivity to the target may be designed using interactive graphics. This combined with the intuition of a medicinal chemist helps in the suggestion of new drug candidates.

Docking[25, 26]

Docking simply refers to the ability to position a ligand in the active or a designated site of a protein and calculates the specific binding affinities. Ligand-protein docking has evolved so remarkably during the past decade that docking single or multiple small molecules to a receptor site is now routinely used to identify ligands. Optimal docking procedures need to be fast, generate reliable ligand geometries, rank the ligand conformation correctly (scoring) and thereby estimate the binding energy. A number of studies have shown that docking algorithms are capable of finding ligands and binding conformations at a receptor site close to experimentally determined structures. These algorithms are equally applicable to the identification of multiple proteins to which a small molecule can bind. The application of this approach may facilitate the prediction of either unknown or secondary therapeutic target proteins are side effects and toxicity of particular drugs. In computational structure-based drug design, the evaluations of scoring functions are the cornerstones to the success of design and discovery. Many approaches have been explored to improve their reliability and accuracy, leading to development of three families of scoring functions. These are force-field-based, knowledge-based and emprical-based.

Scoring function

Scoring functions are normally parameterized (or trained) against a data set consisting of experimentally determined binding affinities between molecular species similar to the species that one wishes to predict.

- **Types**

1. Force field based - Force-field affinities are estimated by summing the strength of intermolecular Van der Waals and electrostatic interactions between all atoms of the two molecules in the complex.

2. Emprical – It is based on counting the number of various types of interactions between the binding partners. Counting may be based on the number of ligand and receptor atoms in contact with each other or by calculating the change in solvent accessible surface area complex compared to the uncomplexed ligand and protein. These interaction terms of the function may include hydrophobic-hydrophobic contacts, hydrophobic-hydrophilic contacts, number of hydrogen bonds, number of rotatable bonds immobilized in complex formation.

3. Knowledge-based (also known as statistical-potentials) – This is based on statistical observations of intermolecular close contacts in large 3D databases which are used to derive "potentials of mean force". This method is founded on the assumption that close intermolecular interactions between certain types of atoms or functional groups that occur more frequently than one would expect by a random distribution are likely to be energetically favourable and therefore contribute favourably to binding affinity.

Absorption, Distribution, Metabolism and Excretion (ADME) analysis[27]

For a drug to be pharmacologically active and exert its action, it should possess favourable pharmacokinetic properties like Absorption, Distribution, Metabolism and Excretion. In the field of drug research and development many promising drugs face failures because they fail to satisfy the ADME parameters.

To rule out the possibility of this, many *in vitro* studies are frequently used to evaluate ADME properties. Some computational methods (*in silico* tools) have been evolved to select the most suitable drug molecules.

In silico modeling serves main functions in predicting ADME properties i.e,

- A deep rooted knowledge in understanding the relationship of ADME parameters and the underlying (drug likeness property) molecular structural features to which it depends on.
- It enhances the interest to the area of posology where it gives information about the drug dosage and frequency. This in turn reflects issues on bioavailability, crossing various biological membranes like brain, ocular and dermal penetration.

These are the essential factors and criteria to look in, for a drug to be pharmacologically active and evolve as a successful clinical candidate in the pharmaceutical research.

Prediction of ADME related parameters

Absorption

To investigate this property *in silico* model uses simple parameters like log D (diffusion coefficient) and polar surface area which are the descriptors for hydrogen bonding capacity and log P (partition coefficient) values. These values should fall under the prescribed values as per the rule of thumb which determines the extent of absorption.

Bioavailability

Factors like size and shape of molecule, lipophilicity and flexibility determines the bioavailability.

Blood Brain Barrier penetration

In order for a drug to cross the blood brain barrier (molecule targeted to brain), as per the rule of thumb, the molecule should have log P values closer to 2 with a molecular mass of <450 Da and/ or with a polar surface area (PSA) <100 Å.

Dermal and Ocular Penetration

For dermal and ocular route it should satisfy the existing parameters like log P (partition coefficient) for aqueous solubility, molecular weight and molecular flexibility.

Metabolism[28]

Various *in silico* approaches exist in evaluating the metabolism namely QSAR and 3D QSAR. Apart from those, computational chemists have updated the structural details in the data bases and tools for predicting metabolism. It also reveals the toxicity related to the molecular fragments formed by metabolic process.

Evaluation of *in silico* toxicity[27]

Toxicity is one of the major criteria to be considered for a molecule to shine as a successful clinical candidate in the pharmaceutical research. About 20-40% of the promising drug candidates fail because of high toxicity. Commercial *in silico* tools estimate toxicity and provide information by the use of QSAR (parameters and descriptors) or scientific literature.

In silico approaches like **OSIRIS** property explorer, predict carcinogenicity, mutagenicity, teratogenicity, immune toxicology, irritation, sensitization etc.

REVIEW OF LITERATURE

Review related to *Solanum virginianum* Linn.,

- Thakkar Atul *et al.*, (2014) determined the alkaloidal value in seven genera of Solanaceae family. They concluded that *Solanum xanthocarpum* and *Nicotiana plumbaginifolia* have the highest alkaloid value. High alkaloid values justify their wide use in traditional system of medicine[29].
- Gaherwal S, Shiv G *et al.*, (2014) studied the antifungal effect of aqueous and hexane leaf extract of *Solanum xanthocarpum* (Kantkari) against *A.niger* and *C.albicans*. The well diffusion and growth inhibition in broth methods were used for evaluating antifungal activity. Results indicate that the aqueous extract was not effective whereas hexane extract showed maximum growth inhibition at 500µg/ml and minimum growth inhibition at 100µg/ml[30].
- Anitha Mary Mathews *et al.*, (2014) studied the ability of aerial parts of *Solanum xanthocarpum* to promote glucose uptake. Diabetes was induced by the administration of STZ. Intraperitoneal administration of nicotinamide in the dose of 230mg/kg prior to the administration of STZ produced partial destruction of β cells that resembled type 2 diabetes. Treatment with the ethanolic extract of aerial parts and fruit of the plant, at the dose of 400mg/kg produced significant reduction in elevated blood glucose level, increase in insulin level and reduction in HbA1c, CK (Creatine Kinase) and LDH (Lactate Dehydrogenase) value by the 28th day of STZ-induced diabetes[31].

- Sridevi Muruhan *et al.,* (2013) evaluated the antioxidant potential of alcoholic leaf extract of *Solanum surattense*. They performed *in vitro* free radical scavenging assays such as hydroxyl radical, hydrogen peroxide and superoxide anion radical scavenging assay, 2, 2-diphenyl-1-picryl hydrazyl radical (DPPH) assay, total antioxidant activity and reducing ability. From the results they have concluded that alcoholic leaf extract of *S.surattense* effectively scavenged free radicals at different concentrations[32].
- Singh S P *et al.,* (2013) evaluated the effect of *Solanum xanthocarpum* seed powder on genital organs and fertility of female albino rats. Different doses of suspension (50, 100 & 150mg/kg/day) were selected for performing anti fertility activity. The genital organ weight of albino rats was reduced significantly ($P<0.05$) after the treatment at 100 and 150mg/kg doses. The higher doses caused histopathological changes in the ovary and uterus leading to 100% control of fertility as no implants were recorded in treated female rats on the 10^{th} day of pregnancy[33].
- Dinanath D Patil (2013) studied the antioxidant activity of ethanol, chloroform and ethyl acetate extract of leaves and stem of *Solanum xanthocarpum* Lam., by DPPH assay using BHT (Butylated Hydroxy Toluene) as the standard. In DPPH scavenging assay, it was shown that the ethanolic extract of leaves and stem shows better antioxidant activity compared to other extracts[34].
- Shraddha K More *et al.,* (2013) evaluated the anti-inflammatory activity of ethanolic extract of whole plant *of Solanum xanthocarpum (*SxE*)* at the maximum dose 100mg/kg p.o. That dose was not sufficient to produce the anti-inflammatory effect in the acute phase; however in chronic

administration, it reduced the proliferative phase of inflammation. The anti-inflammatory activity after chronic administration of SxE was found to be insignificant, which may be due to the low levels of phytochemicals present in whole plant as compared to fruits alone. So they have recommended fruits instead of whole plant for the treatment of inflammation[35].

- Patel P K *et al.,* (2013) studied the antiurolithiatic activity of methanolic extract of fruits of *S. xanthocarpum (*SXME*) in rats*. Urolithiasis was induced by the administration of ethylene-glycol. Different doses (100, 200 and 400mg/kg p.o.) were chosen for the study. Cystone (750mg/kg, p.o.) served as a standard. Administration of SXME reduces the kidney weight by decreasing inflammation and increasing excretion of crystalline components. It reduced and prevented the growth of urinary stones by diuresis, antioxidant activity and maintaining balance between stone promoter and inhibitor constituents[36].
- Ramesh K Gupta *et al.,* (2011) investigated the hepatoprotective potential of 50% ethanolic fruit extract of different doses of *Solanum xanthocarpum* (SXE, 100, 200 or 400mg/kg B.W. for 14 days). Biochemical parameters were used for assessing hepatoprotective activity. Results demonstrated that the treatment with SXE significantly ($P<0.05$- <0.001) and dose dependently prevented chemically induced increase in serum levels of hepatic enzymes and significantly (up to $P<0.001$) reduced the lipid peroxidation in the liver tissue and restored activities of defence antioxidant enzymes GSH, SOD and catalase to normal levels[37].

Review related to other Solanum species

- **Kateregga J N *et al.*, (2015) have reported the** antihyperlipidemic potential of the ethanolic extract of ***Solanum*** *melongena. Hyperlipidemia was induced in* by feeding high fat diet for 3 weeks. The extract was then administered orally at different doses (250 and 500mg/kg/day BW). Atorvastatin (4mg/kg) and distilled water, administered orally, were used as positive and normal control respectively. There was a significant ($P<0.05$) increase in serum total cholesterol and triglycerides in negative control group. The extract, at all doses, produced significant weight reduction in all treatment groups when compared to the normal control group. The 500mg/kg dose of the ethanolic extract of ***Solanum*** *melongena* had the greatest ($P<0.05$) antihyperlipidemic activity[38].
- Tamegnon Victorien Dougnon *et al.,* (2014) have reported on the antihyperlipidemic potential of leaves and fruits of *Solanum macrocarpon*. Hyperlipidemia was induced by Triton X-100 at a single dose of 150mg/kg BW, intraperitoneally. 72 hours after induction, powder of leaves and fruits of *Solanum macrocarpon* were administered daily via oral route for 7 days at different doses (400 and 800mg/kg BW). The biochemical parameters were determined 24 hours later. Treatment with *Solanum macrocarpon* showed a reduction in all hyperlipidemia parameters like TC, TG, VLDL, LDL and an increase in HDL. The mean differences were all statistically significant ($P<0.05$) with the exception of LDL ($P=0.157$)[39].

Review related to models used for evaluation of antihyperlipidemic activity[40-42]

- Cholesterol diet induced hyperlipidemia
- Triton X- 100 induced hyperlipidemia

High fat diet induced hyperlipidemia is one of the most commonly used models for the evaluation of antihyperlipidemic activity. The antihyperlipidemic activity of various plants was carried out by utilizing Cholesterol diet induced hyperlipidemia method. Some of the plants that have been evaluated for antihyperlipidemic activity using Cholesterol diet induced hyperlipidemia model are *Tinospor acordifolia*[43], *Terminalia paniculata*[44], *Cyclocarya paliurus*[45].

Review of *in silico* work

Molecular docking is one of the most frequently used methods in structure-based drug design, due to its ability to predict the binding-conformation of molecule ligands to the appropriate target binding site. Characterization of the binding behaviour plays an important role in rational design of drugs as well as to elucidate fundamental biochemical processes. The associations between biologically relevant molecules such as proteins, nucleic acids, carbohydrates and lipids play a central role in signal transduction. Furthermore, the relative orientation of the two interacting partners may affect the type of signal produced (eg. agonism vs antagonism)[46].

There are various softwares available for docking. They are, Dock, Auto dock, Argus lab, Glide, Gold, Maestro, etc. Glide has been optimized for docking accuracy and database enrichment over a wide range of systems. In order to dock ligands in a

reasonable time, the receptor is treated rigidly. This introduces some sensitivity to the particular receptor conformation used for docking. Glide softens the active site (via vdW scaling) in order to compensate for the lack of receptor flexibility. As long as there are not significant receptor changes upon binding ligands, this generally is sufficient for screening ligands.

If there are induced-fit effects upon binding ligands, however, using a single receptor conformation may penalize classes of actives that bind well to alternative conformations of the receptor. In such cases, it can be useful to perform ensemble docking (i.e., docking to mulitple receptor conformations, with optional Glide Score shifts to account for receptor reorganization energy)[47].

Zhiyoung Zhou *et al.,* studied the Comparative performance of several flexible docking programs and scoring functions: Enrichment studies for a diverse set of pharmaceutically relevant targets. They concluded that The Glide XP methodology is shown to consistently yield enrichments superior to the two alternative methods, while GOLD outperforms and DOCK on average. The study also shows that docking into multiple receptor structures can decrease the docking error in screening a diverse set of active compounds[48].

Bioinformatics tools like molecular docking experiments, which involve study and analysis of ligand-receptor interactions, play important role in identifying molecular targets for different ligands. Novel molecular targets for antihyperlipidemic drugs have been periodically reviewed[49]. There are various molecular targets for

antihyperlipidemic activity. Each molecular target has been individual mechanism of action. Molecular targets for antihyperlipidemic activity are

- Niemann Pick C1 like 1 protein (NPC1L1)[50] - Reduces the absorption of cholesterol
- ATP citrate lyase (ACL)[51] - Supply Ach-Co-A which important for cholesterol biosynthesis
- C-reactive protein (CRP)[52] - Damages LDL
- Lanosterol 14α- demethylase (LDM)[53] - Catalyse the cholesterol biosynthesis
- Squalene synthase (SqS)[54] - Key cholesterol precursor
- Farnesoid X-receptor (FXR)[55] - Cholesterol metabolism

The molecular target NPC1L1 was chosen for docking some of the already isolated compounds of *Solanum virginianum* Linn., NPC1L1 is a gene associated with NPC1 which mutation results in Niemann-Pick disease. It codes for Niemann-Pick C1-like protein 1, found on the gastrointestinal tract epithelial cells as well as in hepatocytes. Specifically, it appears to bind to a critical mediator of cholesterol absorption[56].

There are various Protein Data Banks for NPC1L1 (3QNT, 3GKH, 3GKI, 3GKJ, 3GCW, 3GCX, 3BPS). The PDB file was selected based on its species, X-ray crystallography or NMR spectroscopy, resolution value, external ligand and presence of co-factors. 3GCX was taken for this study.

The characteristic features for 3GCX[57] are

Species - Homosapiens

Resolution - 2.7Å

External ligands - 1

The docked molecules are screened *in silico* using **Molinspiration Cheminformatics Software** to evaluate drug likeness. Toxicity screening is done *in silico* using **OSIRIS** property explorer. It is web based software available on the Organic Chemistry Portal. Using this prediction tool, mutagenicity, tumorogenicity, skin irritancy and reproductive effects can be calculated.

AIM AND OBJECTIVE

The literature review indicates that Solanum species offers a good potential for antihyperlipidemic activity. From the literature review it is clear that no scientific work has so far been carried out on the antihyperlipidemic potential of the fruits of *Solanum virginianum* Linn.,

The aim and objective of the present study is

- To evaluate of *in vivo* antihyperlipidemic potential of ethanolic extract of fruits of *Solanum virginianum* Linn., using the Cholesterol diet induced hyperlipidemia model in adult Wistar rats.

Since *in silico* studies are commonly done to investigate the ADME properties, toxicity parameters and mechanism of action of various potential drug molecules, the aim of this study is also

- To establish the antihyperlipidemic activity, ADME properties and toxicity of some of the already isolated selected compounds of *Solanum virginianum* Linn., using *in silico* studies.

PLANT PROFILE




Figure 2: *Solanum virginianum* Linn.,

Plant introduction[58, 59, 8, 9]

Biological name : *Solanum virginianum* Linn.

Synonyms : *Solanum surattense, Solanum mccanni,*

Solanum xanthocarpum Schard. & Windl.,

Family : Solanaceae

Vernacular names[58, 59]

Tamil	:	Kandangattari, udavani
Hindi	:	Chotikateri, Rengani
Telugu	:	Nelamulaka
English	:	Yellow-berried night shade, Febrifuge plant
Bengali	:	Kantikari
Sanskrit	:	Kantakari, Nidigadhika

Taxonomic classification[58, 59]

Botanical name	:	*Solanum virginianum* Linn.
Kingdom	:	Plantae
Division	:	Magnoliophyta
Class	:	Magnoliopsida
Order	:	Solanales
Family	:	Solanaceae
Genus	:	*Solanum*
Species	:	*virginianum*

Geographical distribution[58, 59]

It is found throughout India, Malaya, Ceylon, Southeast Asia and Polynesia. In India, it is mainly grown in Uttar Pradesh, Bihar, Punjab, Uttaranchal, West Bengal, Assam and other North-Eastern States.

Morphology[60, 61]

It is a very prickly diffuse bright green perennial herb, 2-3m high, somewhat woody at the base, stems are zigzag, branches are numerous, prickles are compressed, straight, yellow, glabrous and shining, often exceeding 1-3cm long.

Leaves: 5-10 leaves, ovate or elliptic, sinuate or sub pinnatified, obtuse or subacute, stellately hairy on both sides, armed on the mid rib and often on the nerves with long yellow sharp prickles, base usually rounded and unequal-sided, petioles 1.3-2.5cm long, stellately hairy and prickly.

Flowers: Extra-axillary few flowered cymes sometimes reduced to a single flower, peduncles short, pedicels short, curved, stellately hairy. **Calyx:** 1.3 cm, long, densely hairy and prickly, tube short, globos. **Corolla:** 2 cm, purple, long, lobes deltoid, acute, hairy outside. **Filaments:** 1.5mm long, glabrous. **Anthers:** 8mm. long, oblong-lanceolate, opening by small pores. **Ovary:** ovoid, glabrous.

Berries: 1.3-2 cm diameter, yellow or white with green veins, surrounded by the enlarged calyx. Seeds: 2.5 mm diameter, glabrous.

Parts used

Whole plant, root, flower, fruit.

Chemical constituents[62- 66]

Plant contains alkaloids, sterols, saponins, flavonoids and their glycosides and also carbohydrates, fatty acids, amino acids etc.

Bark: caffeic acid, methyl caffeate, solasonine, solasurine.

Root: apigenin, coumarin, esculetin, esculin, scopoletin.

Seed: glucose, linoleic acid, lysine, leucine, solanacarpine, solanacarpigenin, stearic acid, α-solamargine.

Fruit: α-solamargine, β-sitosterol, campesterol, cycloartanol, esculetin, esculin, lupeol, solasodine, solasonine, scopoletin, solanine, solanidine, stigmasterol, sitosteryl glucoside, stigmasteryl glucoside, tomatidenol.

Traditional uses[67, 68, 9]

- The plant has been useful in cold, cough, fever, skin diseases, cardiac disorders and worm infections.
- It is also known to improve strength and immunity.
- It is used in the treatment of asthma and chronic respiratory disorders.

PLAN OF WORK

- Collection of fruits of *Solanum virginianum* Linn.,
- Authentication of plant material.
- Processing of fruits and extraction of seeds with 90% ethanol.
- *In vivo* antihyperlipidemic activity – Cholesterol diet induced hyperlipidemia model
    - Changes in body weight
    - Biochemical parameters
- *In silico* antihyperlipidemic studies on selected isolated compounds of the plant of *Solanum virginianum* Linn.,
    - Toxicity studies
    - Docking
    - Drug likeness

MATERIALS AND METHODS

I. *IN VIVO* STUDIES

1. Collection and identification of plant material

The fruits of *Solanum virginianum* Linn., were collected from the waste lands in Krishnagiri district, Tamilnadu in the month of August, 2015. The plant was identified and authenticated by Prof. Sasikala Ethirajulu, Botanist, Siddha Central Research Institute, Arumbakkam, Chennai-600106.

2. Preparation of plant extract[69]

Chemicals

Analytical grade of 90% ethanol was bought from Microfine Chemicals, Chennai.

Extraction

The fruits were washed with tap water, shade dried at room temperature and then subjected to size reduction to a coarse powder by using wiley mill. The powdered fruit material was packed in a Soxhlet apparatus and extracted with 90% ethanol. The extraction was continued until the color of the solvent in the siphon tube became colorless. The ethanolic extract was concentrated in a rotary evaporator and this concentrated ethanolic extract of fruits of *Solanum virginianum* Linn., (EEFSV) was used for the *in vivo* studies.

3. *In* vivo screening

3.1 Experimental animals

Healthy wistar rats (150-200g) were procured from Animal Experimental Laboratory, Madras Medical College, Chennai-03. The study was approved by Institutional Animal Ethics Committee which is certified by the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), India.

Approval Number: 04/243/CPCSEA dated 10.08.2015

3.2 Maintenance of animals[70]

The animals were kept in clean and dry polypropylene cages with stainless steel top grill having facilities for pelleted food and water. The animals were maintained in a well ventilated animal house in 12 hours and 12 hours dark cycle at a temperature of $28^{o}C \pm 2^{o}C$ and they were acclimatized to laboratory conditions for 10 days prior to the commencement of the experiment. The animals were fed with standard pellet diet and water *ad libitum*. All animal experiments were performed according to the ethical guidelines suggested by Institutional Animal Ethics Committee (IAEC). Paddy husk was used as bedding material and changed twice a week.

3.3 Acute toxicity studies[71, 9]

Acute toxicity study has been already performed on the fruits of *Solanum virginianum* Linn., Fruits of *Solanum virginianum* Linn., were administered orally as a single dose to mice at different dose levels of 250, 500, 1000 and 2000mg/kg BW.

Animals were observed periodically for the symptoms of toxicity and death within 24h and then daily for 14 days. Fruits of *Solanum virginianum* Linn., produced no mortality at 2000mg/kg. Hence $1/10^{th}$ (200mg/kg) and $1/5^{th}$ (400mg/kg) of this dose was chosen for the antihyperlipidemic study.

3.4 Evaluation of anti hyperlipidemic activity

The model used to evaluate the anti hyperlipidemic activity was Cholesterol diet induced hyperlipidemia in rats.

Cholesterol –diet induced hyperlipidemia in rats[38, 40-45]

Cholic acid and Cholesterol powder were bought from Microfine Chemicals, Chennai. All the chemicals used in the study were of analytical grade.

- **Procedure**

Hyperlipidemia was induced in rats by administration of cholesterol diet for 30 days. Cholesterol diet consists of Cholesterol 12%, Cholic acid 1%, Sucrose 40% and Coconut oil 10%. All the animals were weighed and divided into five groups, each group containing six animals. The details of the grouping are given in **Table 3**.

Table 3: Study design

S. No	Group (n=6)	Name of the group	Treatment schedule
1	I	Normal control	Rat chow diet for 30 days.
2	II	Hyperlipidemic control	Cholesterol diet for 30 days.
3	III	Standard	Cholesterol diet for 30 days + Atorvastatin 2mg/kg p.o from 16^{th} to 30^{th} day.
4	IV	Low dose	Cholesterol diet for 30 days + EEFSV 200mg/kg p.o from 16^{th} to 30^{th} day.
5	V	High dose	Cholesterol diet for 30 days + EEFSV 400mg/kg p.o. from 16^{th} to 30^{th} day.

The blood samples were collected on 0, 15th and 30th day of the experiment from the retro orbital sinus using glass capillary. The blood was allowed to clot for 30 minutes at room temperature. The clear serum was separated by centrifugation at 2500 rpm for 10 minutes and used for the determination of biochemical parameters.

- **Parameters evaluated**

  - **Changes in body weight**

The body weight of each animal in every group were recorded on 0, 15th and 30th day of study period and the changes in body weight were noted.

  - **Biochemical analysis**

The serum was subjected to the following evaluation

- Total Cholesterol (TC)
- Triglycerides (TG)
- High density lipoprotein (HDL)
- Very Low density lipoprotein (VLDL)
- Low density lipoprotein (LDL)

  - **Statistical analysis**

The results of the biochemical estimations were presented as mean ± SD of six animals in each group. Total variations, present in a set of data were estimated by One Way Analysis Of Variance (ANOVA). P value of <0.05 was considered statistically significant.

II. *IN SILICO* STUDIES

Review of literature showed that fruits of *Solanum virginianum* Linn., have the phytochemicals like alkaloid, phenolic compounds, flavonoid, glycoalkaloid, sapogenin, coumarin, steroidal alkaloid, glycoside, carbohydrate, triterpenoid, steroids, fatty acids, amino acids, etc[60]. Alkaloid, phenolic compounds, flavonoid, glycoalkaloid, sapogenin, coumarin, steroidal alkaloids were taken up for *in silico* toxicity, docking and drug likeness studies.

Isolated compounds taken for this study include

1. α- Solamargine - Alkaloid
2. Tomatidenol - Alkaloid
3. Esculin - Coumarin
4. Esculetin - Coumarin
5. Scopoletin - Coumarin
6. Apigenin - Flavonoid
7. Carpesterol - Glycoalkaloid
8. Methyl caffeate - Phenolic
9. Caffeic acid - Phenolic
10. Coumarin - Phenolic
11. Diosgenin - Sapogenin
12. Solanidine - Steroidal alkaloid
13. Solanine - Steroidal alkaloid
14. Solasodine - Steroidal alkaloid
15. Solasonine - Steroidal alkaloid

A. *In silico* toxicity prediction

Toxicity screening is done *in silico* using **OSIRIS** property explorer. It is a web based software available on the Organic Chemistry Portal. Using this prediction tool, mutagenicity, tumorogenicity, skin irritancy and reproductive effects can be calculated. The prediction properties depends on a precompiled set of structure fragment that gives rises to toxicity alerts, if they are found in the structure currently drawn. These fragment lists is created by rigorously shredding all compounds in the data base known to be active in a certain toxicity class. During the shredding any molecule is first cut at every rotatable bonds leading to a set of core fragments[76]. OSIRIS software is used to calculate various drug relevant properties of chemical structures. The results are color coded. The green color represents that the compound is non-toxic. Yellow and red color indicates moderate and severe toxicity of the chemical respectively[49].

B. Docking

In this study, Glide (Grid based Ligand Docking with Energetics) program was used for screening the isolated compounds. Glide automatically searches for favorable interactions between ligand molecule and the receptor in different conformations. Docking procedure using Glide includes the following steps,

1. Protein preparation
2. Receptor grid generation
3. Ligand preparation
4. Ligand docking
5. Visualizing docking poses

1. Protein preparation

Protein data bank (PDB) file, which is the crystallized structure of the receptor/ protein is imported from Protein data bank with the following **PDB Id: 3GCX**, resolution **2.7Å**, preprocessed involving addition of hydrogen, assigning bond order, finding overlaps, creating zero order bond to metals, creating disulfide bonds, filling missing side chains and loops using prime option. The water molecules, co-factors and unwanted chains were deleted. The energy minimization was done to make it ready for grid generation. The PDB file was selected based on its species, X-ray crystallography or NMR spectroscopy, resolution value, external ligand and presence of co-factors[72].

2. Receptor grid generation

After the preparation of protein, the grid has to be generated which is the critical process. It includes defining the active site in the protein (receptor). The prepared protein file was loaded into the workspace. The active site residues were found and picked and the length for docking the ligand to the protein is given as 10Å. The grid was generated by pressing "start" in the grid generation tab. The grid output file obtained as zip file format was utilized for further docking process.

3. Ligand preparation

Ligand preparation process consists of a series that include conversions, applying corrections to the structures drawn, generating variations on the structure, eliminating unwanted structures and optimizing the structures. Variations on the structure can be made by addition of hydrogen atoms, removal of unwanted molecules, neutralizing changed groups. The structure can be optimized by generating ionization states, generating tautomers, filtering their structure on the basis of Lipinski's rule of five.

4. Ligand docking

After the generation of grid, the prepared ligands were docked to see the interaction with the active site of the protein. There were hydrophobic, hydrophilic and Van der Waal's interaction. The strength of the interaction was different ligand molecules. During the docking procedure, conformation of the ligand was retained and extra precision (xp) mode was selected. In this procedure, the following constraints like active site and rotatable groups have been checked[73].

5. Visualization of the docking poses

Once the molecules were docked, then they were visualized for interactions, score and some other parameters like log P value and ionization value. There were interactions like hydrogen bonding, hydrophobic interaction, Van der Waal's interaction between the receptor and the ligand. Based on the interaction and score obtained, the molecules were categorized into hit and flop.

C. *In silico* screening of drug likeness

For a drug to be pharmacologically active and exert action it should posses pharmacokinetic properties like absorption, distribution, metabolism and excretion. Many drug failures occur due to unfavorable ADME properties in the field of drug research and development. This has to be ruled out earlier in the process of drug discovery. Some computational methods (*in silico* tools) have been evolved to investigate the most suitable drug molecules before synthesis.

Lipinski's rule of five also known as the **Pfizer's rule of five,** is a rule to evaluate drug likeness. It is used to predict whether a molecule is likely to be orally bio-available or to evaluate drug likeness[74].

The designed and docked molecules are screened *in silico* using **Molinspiration Cheminformatics Software** to evaluate drug likeness. This tool is quick and easy to use. It can be accessed online for calculation of important molecular properties such as log P, polar surface area, number of hydrogen bond donors and acceptors as well as prediction of bioactivity score for the most important drug targets like GPCR ligands, kinase inhibitors, ion channel modulators, nuclear receptors[75].

RESULTS AND DISCUSSION

I. *In vivo* anti hyperlipidemic activity

A. Effect of ethanolic extract of fruits of *Solanum virginianum* Linn., on body weight of animals

The results are tabulated in **Table 4**. Hyperlipidemic rats showed an increase in body weight whereas the weight of the control rats remained the same. Administration of Atrovastatin and ethanolic extract of fruit of *Solanum virginianum* Linn., for 15 days significantly reduced the weight and brought back the BW towards normal.

Table 4: Effect of Cholesterol diet on body weight and the effect of Atorvastatin and EEFSV on the weight of hyperlipidemic rats

Group	Body Weight (g)		
	Induction period		Treatment period
	0 Day	15th Day	30th Day
I	181.5±0.957	181.5±1.258	182.16±1.067
II	179.83±1.343	200.33±0.942[a]	220.16±1.213[b]
III	180.83±1.34	199.83±1.863[a]	184.5±1.384[b]
IV	181.16±1.067	200.66±1.374[a]	186.83±1.674[b]
V	179.83±1.462	201.16±1.674[a]	184.66±1.247[b]

Values are as expressed as mean ± SD (n=6)

a- P <0.001 compared with control
b- P<0.001 compared with disease control

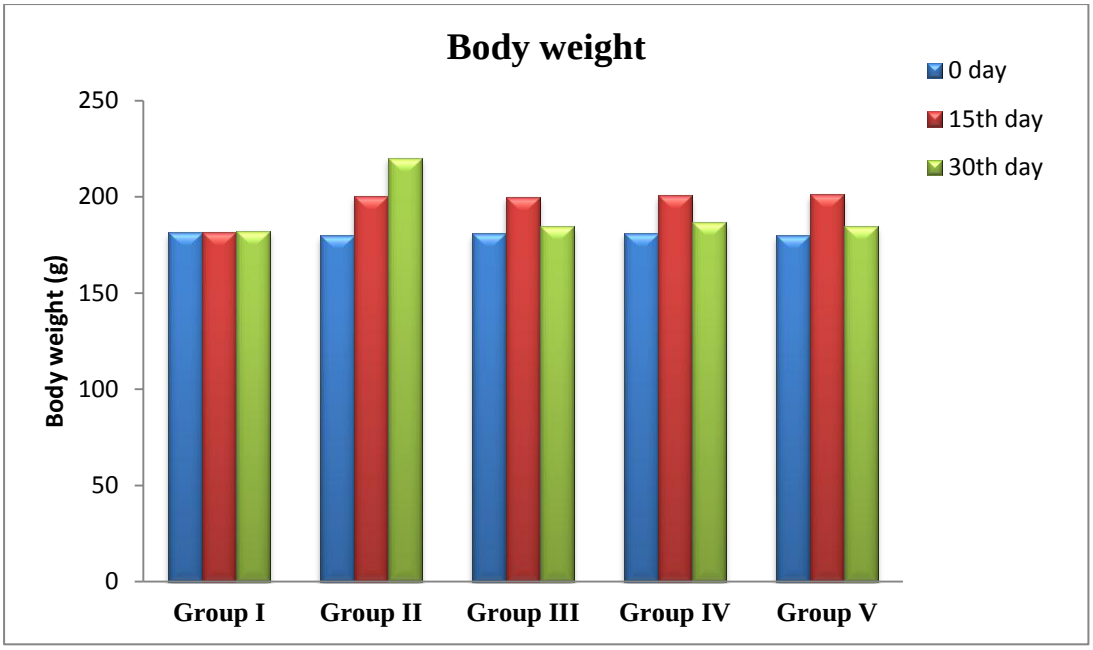


Figure 3: Effect of EEFSV on body weight

B. Biochemical analysis

1. Effect of ethanolic extract of fruits of *Solanum virginianum* Linn., on Total Cholesterol level in hyperlipidemic rats

The results are tabulated in **Table 5**. Hyperlipidemic rats showed an increase in Total Cholesterol whereas the Total Cholesterol of the control rats remained the same. Administration of Atrovastatin and ethanolic extract of fruit of *Solanum virginianum* Linn., for 15 days significantly reduced the levels and brought back Total Cholesterol towards normal.

Table 5: Effect of Cholesterol diet on TC and the effect of Atorvastatin and EEFSV on TC of hyperlipidemic rats

Group	Total Cholesterol (mg/dL)		
	Induction period		Treatment period
	0 Day	15th Day	30th Day
I	91.33±5.15	90±3.87	91±5.09
II	89.33±5.79	170±5.80 [a]	252.83±4.37 [b]
III	87.16±6.09	170.5±5.64[a]	100.66±3.29[b]
IV	86.66±5.67	168±5.62[a]	115.33±3.63[b]
V	89.66±6.47	169.66±5.21[a]	103.83±2.26[b]

Values are as expressed as mean ± SD (n=6)

a- P<0.001 compared with control

b- P<0.001 compared with disease control

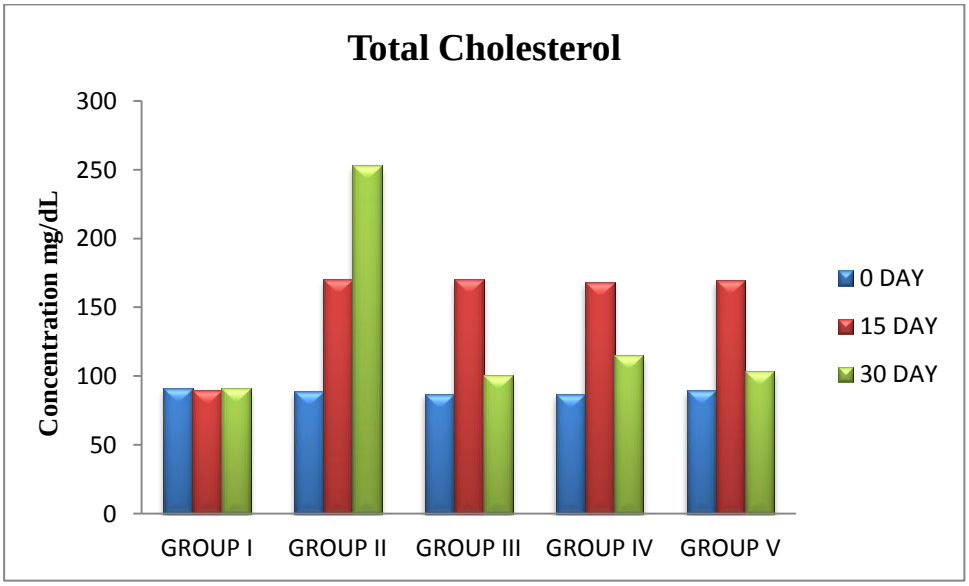


Figure 4: Effect of EEFSV on Total Cholesterol

2. Effect of ethanolic extract of fruits of *Solanum virginianum* Linn., on Triglyceride level in hyperlipidemic rats

The results are tabulated in **Table 6**. Hyperlipidemic rats showed an increase in Triglycerides whereas the Triglycerides of the control rats remained the same. Administration of Atrovastatin and ethanolic extract of fruit of *Solanum virginianum* Linn., for 15 days significantly reduced the levels and brought back Triglycerides towards normal.

Table 6: Effect of Cholesterol diet on TG and the effect of Atorvastatin and EEFSV on TG of hyperlipidemic rats

Group	Triglycerides (mg/dL)		
	Induction period		Treatment period
	0 Day	15th Day	30th Day
I	69±4.50	68.66±3.94	68.5±3.59
II	70±3.05	150.66±4.74[a]	211.13±7.01[b]
III	69.83±2.54	149.66±6.15[a]	74.66±2.92[b]
IV	67.66±5.52	148.66±5.59[a]	96.66±3.72[b]
V	69.33±4.81	149.83±5.11[a]	84±2.23[b]

Values are as expressed as mean ± SD (n=6)

a- P<0.001 compared with control

b- P<0.001 compared with disease control

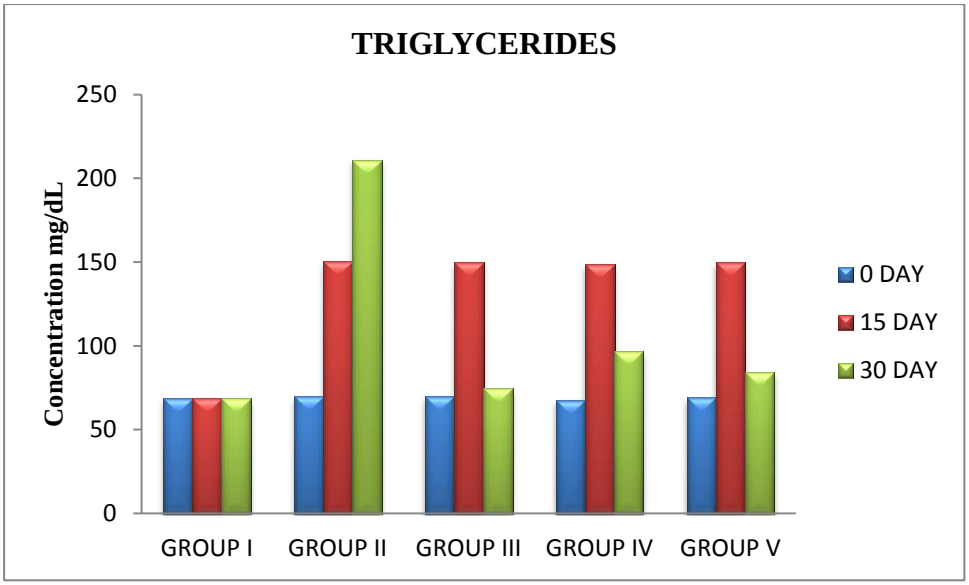


Figure 5: Effect of EEFSV on Triglycerides

3. **Effect of ethanolic extract of fruits of *Solanum virginianum* Linn., on HDL level in hyperlipidemic rats**

The results are tabulated in **Table 7**. Hyperlipidemic rats showed decrease in HDL whereas the HDL of the control rats remained the same. Administration of Atrovastatin and ethanolic extract of fruit of *Solanum virginianum* Linn., for 15 days significantly increase the levels of HDL and brought back HDL towards normal.

Table 7: Effect of Cholesterol diet on HDL and the effect of Atorvastatin and EEFSV on HDL of hyperlipidemic rats

Group	HDL (mg/dL)		
	Induction period		Treatment period
	0 Day	15thDay	30th Day
I	25.83±2.26	25.16±2.11	25.5±1.97
II	25.5±2.98[a]	19.5±2.81[a]	13.66±1.49[b]
III	25±1.91[b]	19.16±1.77[a]	26.16±1.95[b]
IV	25±3.26[b]	19.83±1.77[a]	22±1.29[b]
V	24.33±3.09[b]	19±2.01[a]	25.83±1.34[b]

Values are as expressed as mean ± SD (n=6)

a- P<0.001 compared with control

b- P<0.001 compared with disease control

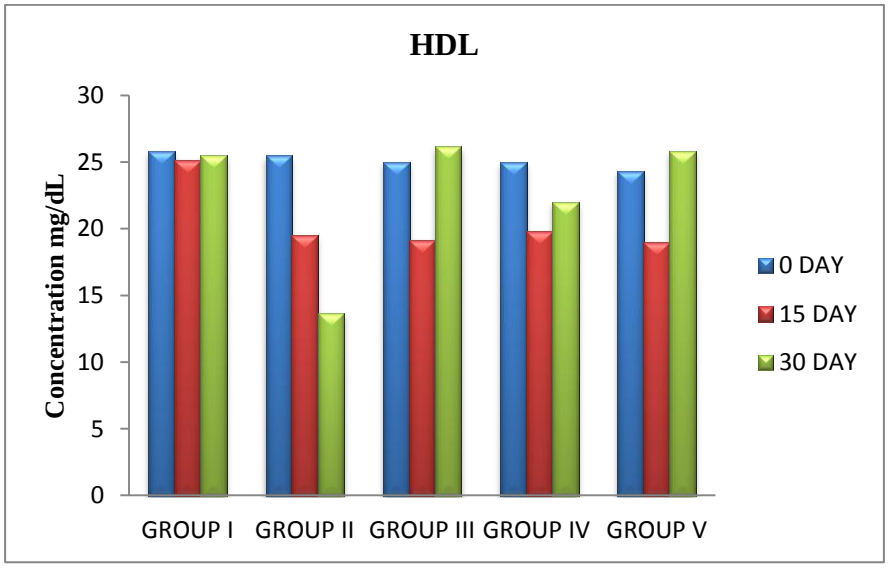


Figure 6: Effect of EEFSV on HDL

4. Effect of ethanolic extract of fruits of *Solanum virginianum* Linn., on VLDL level in hyperlipidemic rats

The results are tabulated in **Table 8**. Hyperlipidemic rats showed an increase in VLDL whereas the VLDL of the control rats remained the same. Administration of Atrovastatin and ethanolic extract of fruit of *Solanum virginianum* Linn., for 15 days significantly reduced the levels and brought back VLDL towards normal.

Table 8: Effect of Cholesterol diet on VLDL and the effect of Atorvastatin and EEFSV on VLDL of hyperlipidemic rats

Group	VLDL (mg/dL)		
	Induction period		Treatment period
	0 Day	15th Day	30th Day
I	13.8±0.90	13.73±0.78	13.7±0.71
II	14±0.61	30.13±0.94[a]	42.26±1.40[b]
III	13.96±0.50	29.93±1.23[a]	14.9±0.58[b]
IV	11.21±4.50	29.73±1.19[a]	19.33±0.74[b]
V	13.86±0.96	29.96±1.02[a]	16.8±0.44[b]

Values are as expressed as mean ± SD (n=6)

a- P<0.001 compared with control

b- P<0.001 compared with disease control

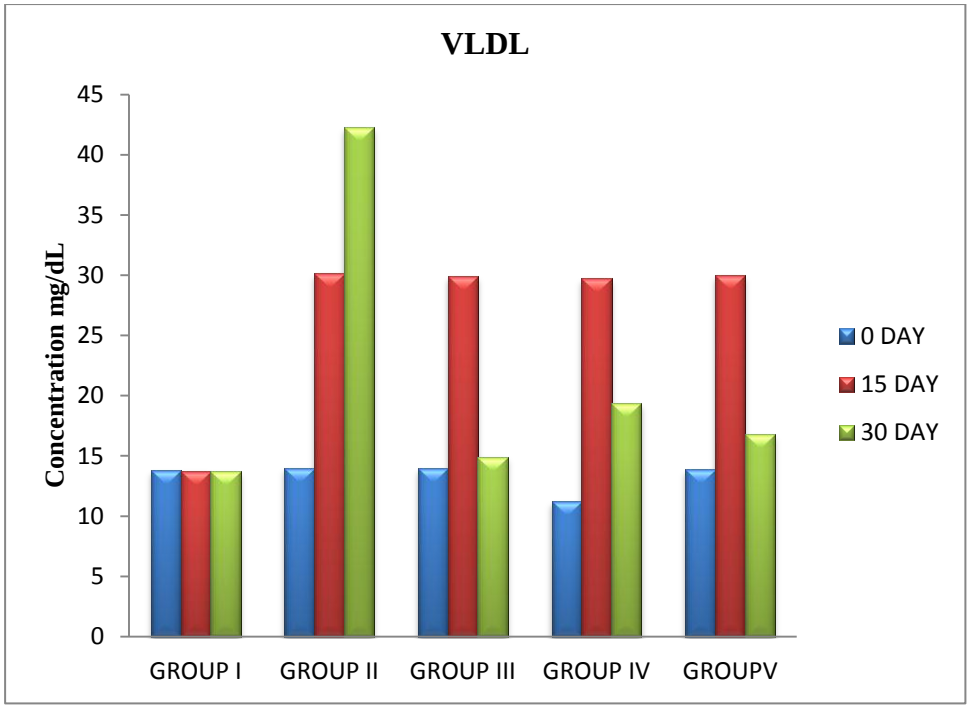


Figure 7: Effect of EEFSV on VLDL

5. Effect of ethanolic extract of fruits of *Solanum virginianum* Linn., on LDL level in hyperlipidemic rats

The results are tabulated in **Table 9**. Hyperlipidemic rats showed an increase in LDL whereas the LDL of the control rats remained the same. Administration of Atrovastatin and ethanolic extract of fruit of *Solanum virginianum* Linn., for 15 days significantly reduced the levels and brought back LDL towards normal.

Table 9: Effect of Cholesterol diet on LDL and the effect of Atorvastatin and EEFSV on LDL of hyperlipidemic rats

Group	LDL (mg/dL)		
	Induction period		Treatment period
	0 Day	15th Day	30th Day
I	53.6±5.89	51.1±5.05	51.5±6.80
II	49.5±7.58[a]	120.36±7.51[a]	196.9±4.67[a]
III	48.2±7.08[b]	125.9±8.92[b]	59.56±5.42[b]
IV	48.11±7.76[b]	118.43±6.20[b]	74±4.25[b]
V	51.46±8.50[b]	120.7±5.89[b]	61.2±3.39[b]

Values are as expressed as mean ± SD (n=6)

a- P<0.001 compared with control

b- P<0.001 compared with disease control

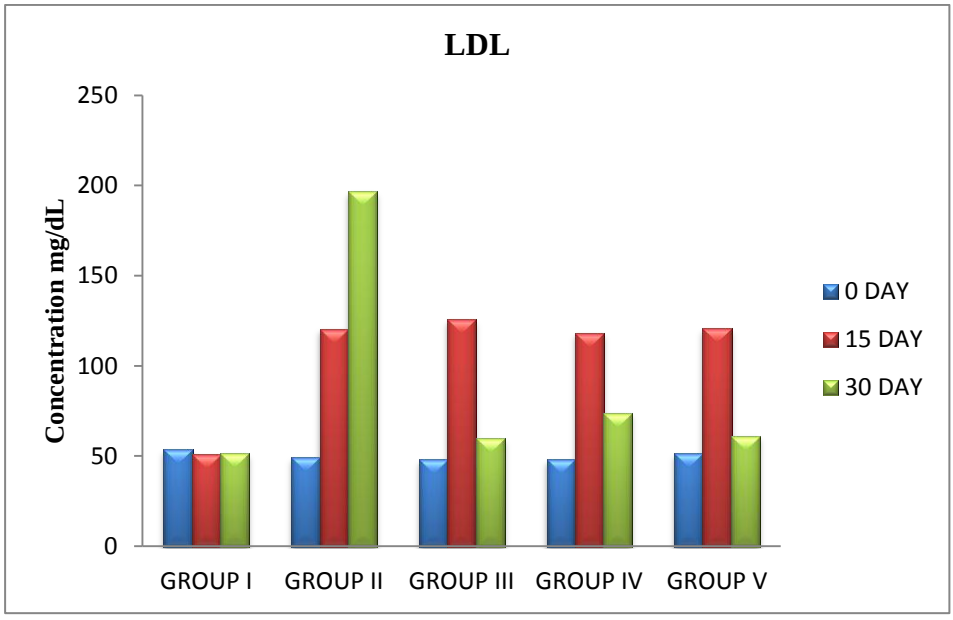


Figure 8: Effect of EEFSV on LDL

II. *In silico* studies

A. Toxicity studies

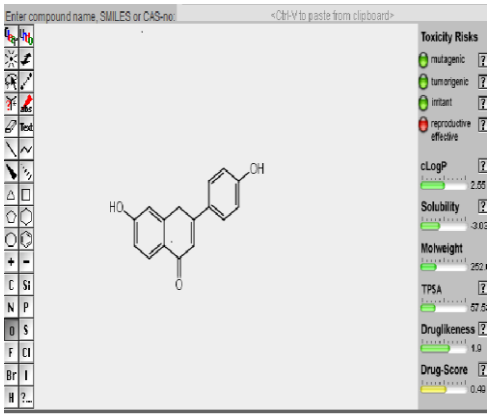
Toxicity is one of the major criteria to be considered for a molecule to shine as a successful clinical candidate in the pharmaceutical research. So the toxicity studies of some of the already isolated molecules of *Solanum virginianum* Linn., were performed. Toxicity was predicted by the **OSIRIS** Property Explorer, the online software of Thomas Sander, Acetelion Pharmaceuticals Ltd., Gewerbestrasse 16 and 4123 Allschwil, Switzerland. Prediction results were valued and color coded. Properties with high risks like mutagenicity, reproductive effect, tumorigenicity and skin irritantcy are shown in red color whereas a green and orange color indicates non- toxic behavior of the drug. Toxicity parameters are tabulated in **Table 10** and **Figure 9.**

Table 10: Prediction of toxicity

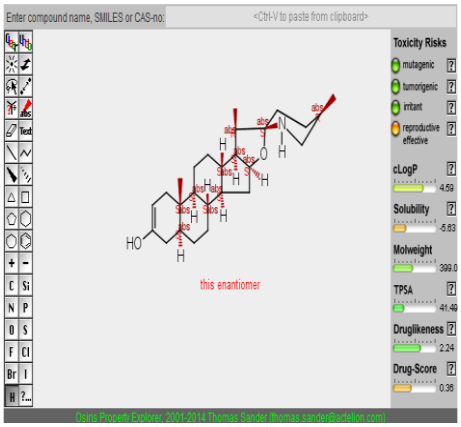
S. No	Molecules	Mutagenicity	Tumerogenicity	Irritant	Reproductive effect
1	Apigenin	-	-	-	+
2	Caffeic acid	+	+	-	+
3	Carpesterol	-	-	-	-
4	Coumarin	+	+	-	+
5	Diosgenin	-	-	-	-
6	Esculetin	-	+	-	-
7	Esculin	-	-	-	-
8	Methyl caffeate	-	-	-	-
9	Scopletin	-	-	-	-
10	α-Solamargine	-	-	-	-
11	Solanidine	-	-	-	-
12	Solanine	-	-	-	-
13	Solasodine	-	-	-	-
14	Solasonine	-	-	-	-
15	Carpesterol	-	-	-	-

- indicates as safe; + indicates as toxic

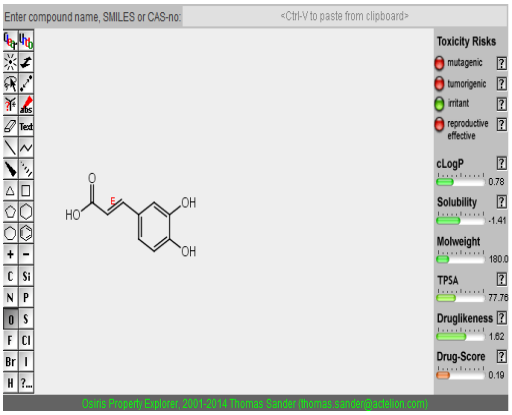
Figure 9 (Part-I): Toxicity of already isolated compounds



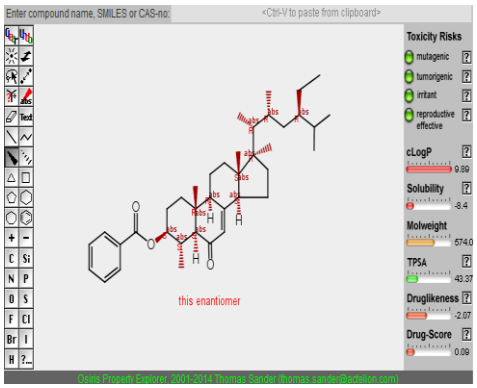
Apigenin



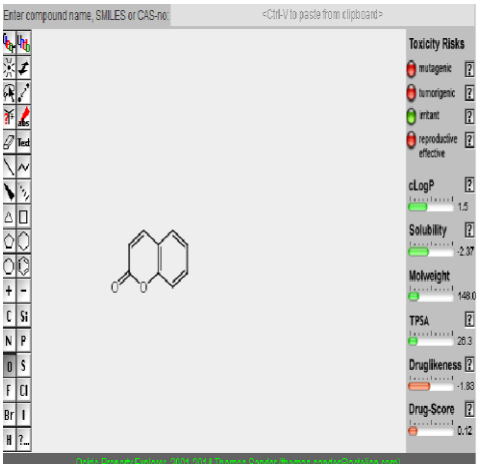
Tomatidenol



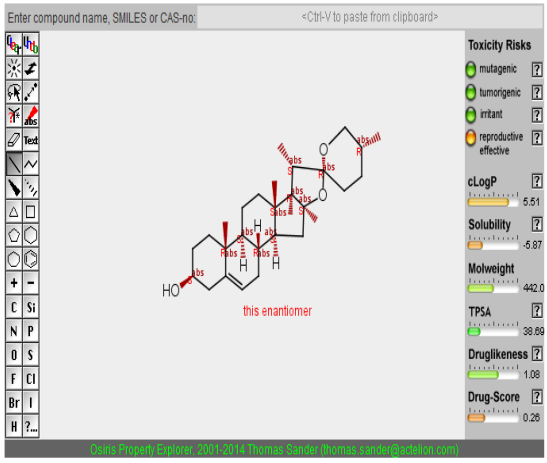
Caffeic acid



Carpesterol

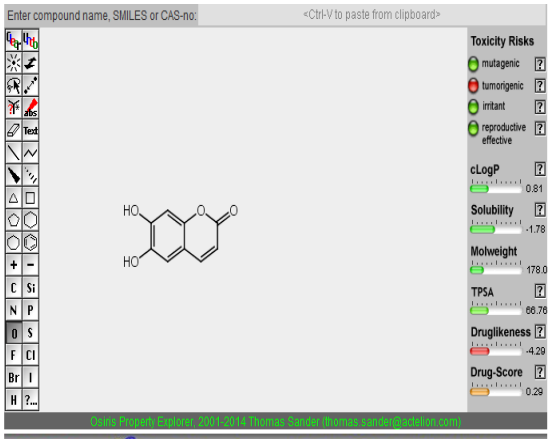


Coumarin

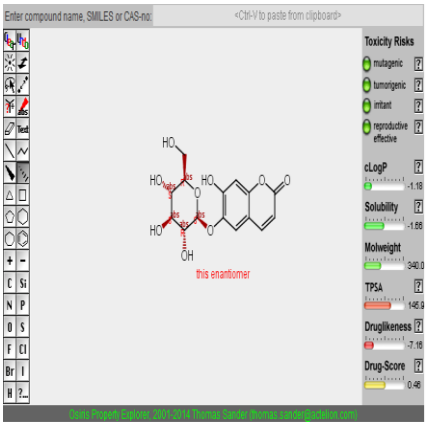


Diosgenin

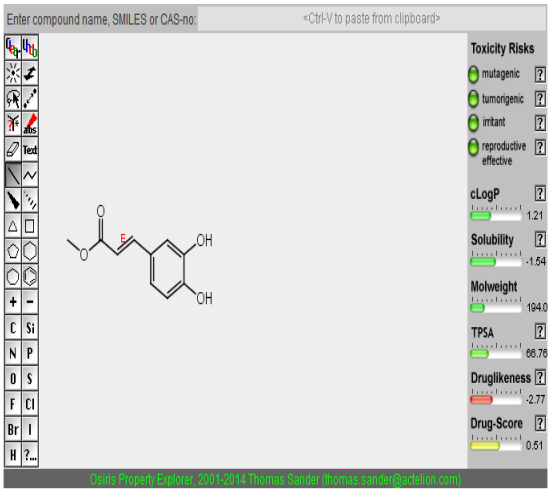
Figure 9 (Part-II): Toxicity of already isolated compounds



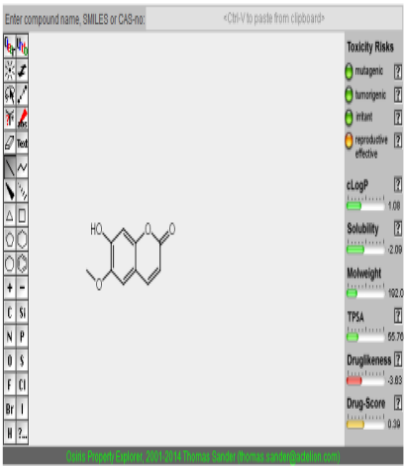
Esculetin



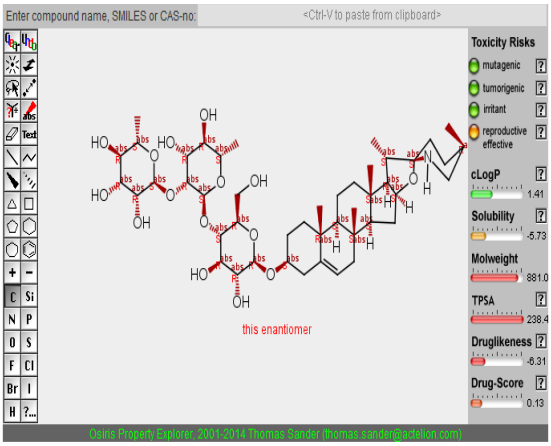
Esculin



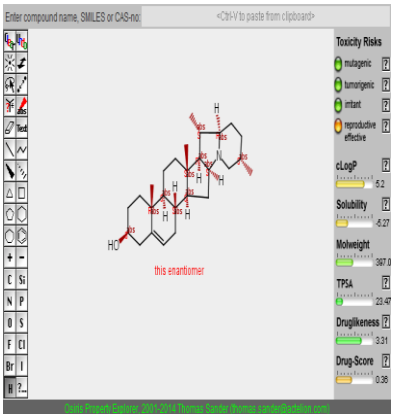
Methyl Caffeate



Scopoletin

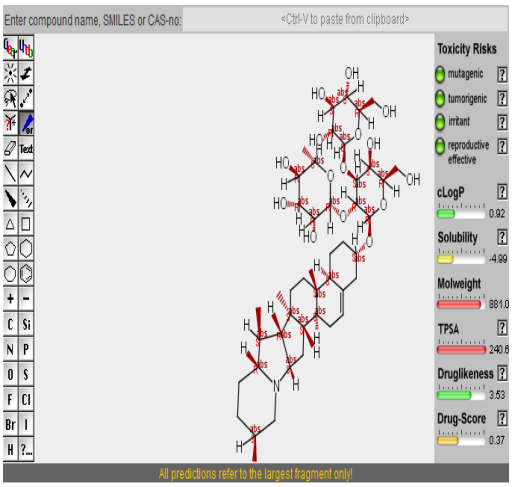


α- Solamargine

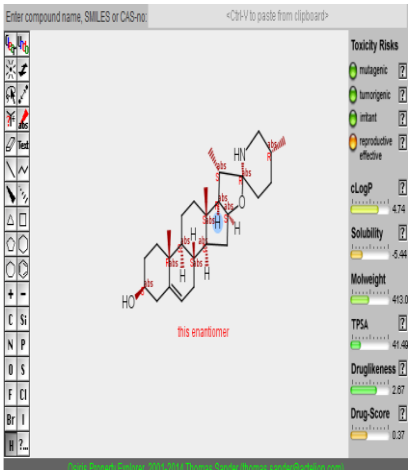


Solanidine

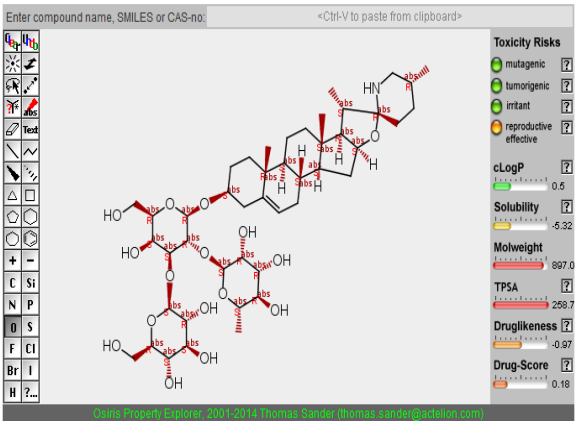
Figure 9 (Part-III): Toxicity of already isolated compounds



Solanine



Solasodine



Solasonine

Among the 15 molecules which were subjected for evaluation 11 molecules were found to be non- toxic. These molecules are Carpesterol, Diosgenin, Esculin, Methyl caffeate, Scopletin, α-Solamargine, Solanidine, Solanine, Solasodine, Solasonine and Tomatidenol.

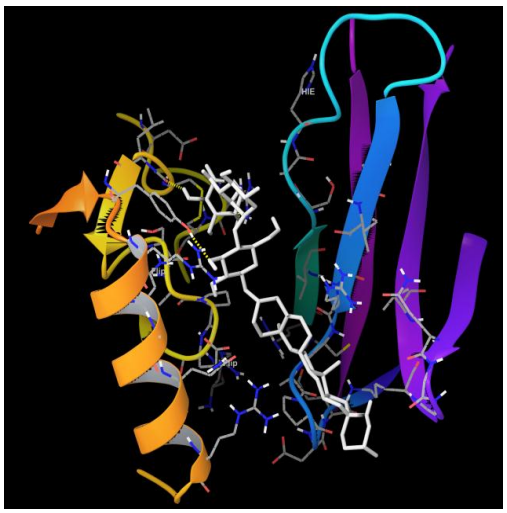
B. Docking

The same 15 compounds were docked against the target 3GCX after the active site on the protein was selected using Sitemap function. For perfect docking of the ligand into the cavity of the protein having active site, extra precision mode molecular docking was executed. During the docking procedure different poses of the ligand were generated. The ligands were docked in different poses. The best docked pose was selected based on the G Score and the interactions between the protein and the ligand. G score values are tabulated in **Table 11 and Figure 10**.

Table 11: Compounds docked against 3GCX

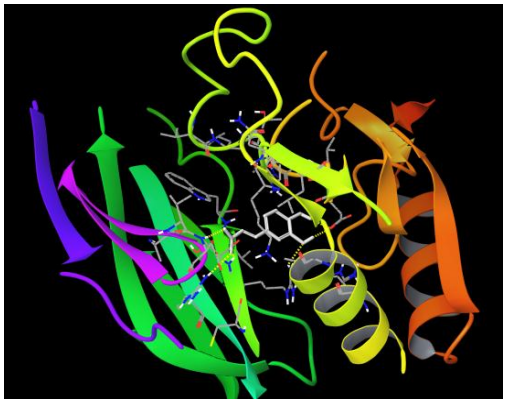
S. No	Ligands	G score	D Score	Lipophilic EvdW	H Bond	Electro
1	Solanine	-11.32	-11.32	-2.53	-7.96	-1.74
2	Esculin	-7.51	-7.51	-2.87	-3.16	-1.08
3	Solasonine	-6.66	-6.64	-3.26	-4.09	-1.05
4	Esculetin	-6.51	-6.5	-2.87	-2.47	-0.67
5	α-Solamargine	-6.24	-4.29	-1.87	-4.16	-0.75
6	Apigenin	-5.84	-5.82	-3.28	-1.47	-0.69
7	Scopletin	-5.5	-5.49	-2.83	-1.62	-0.55
8	Caffeic acid	-5.3	-5.3	-1.26	-1.66	-0.74
9	Methyl caffeate	-4.6	-4.64	-1.98	-1.53	-0.76
10	Coumarin	-4.06	-4.06	-2.71	-0.7	-0.15
11	Solanidine	-3.52	-3.52	-2.14	-0.98	-0.57
12	Diosgenin	-1.18	-1.18	-1.56	0	-0.01
13	Solasodine	-0.88	-0.86	-0.79	0	-0.35
14	Carpesterol	0.12	0.12	-1.76	-0.44	-0.18
15	Tomatidenol	0.79	0.81	-1.39	0	-0.24
16	Atorvastatin	-5.38	-5.38	-3.95	-2.26	-0.94

Figure 10 (Part-I): Compounds docked against 3GCX



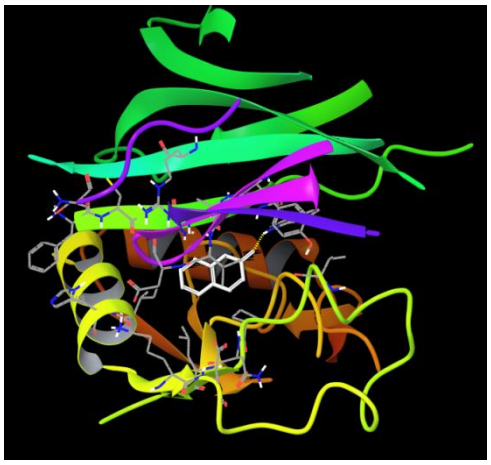
Solamargine

Apigenin

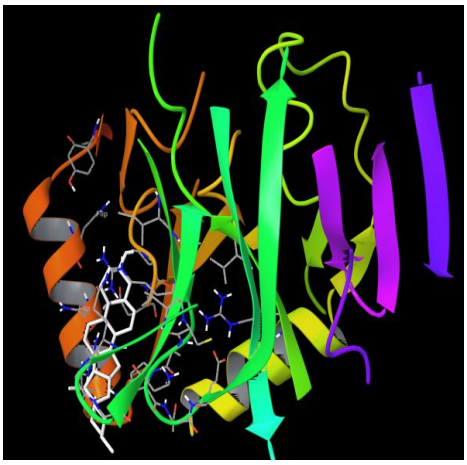


Caffeic acid

Carpesterol

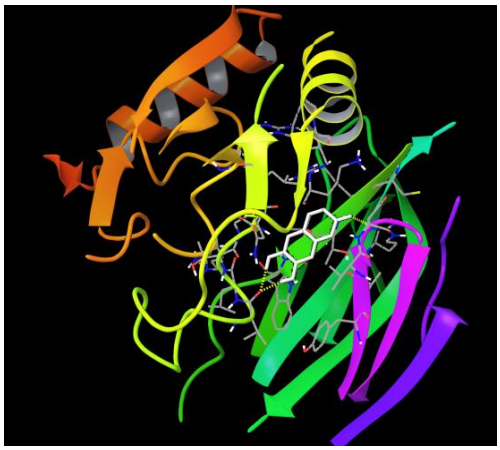


Coumarin

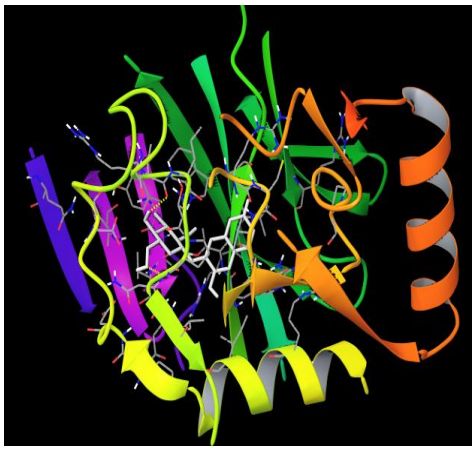


Diosgenin

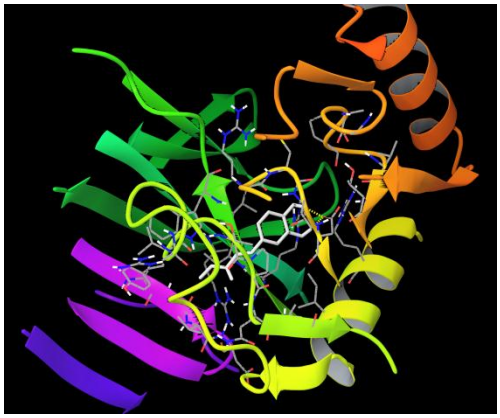
Figure 10 (Part-II): Compounds docked against 3GCX



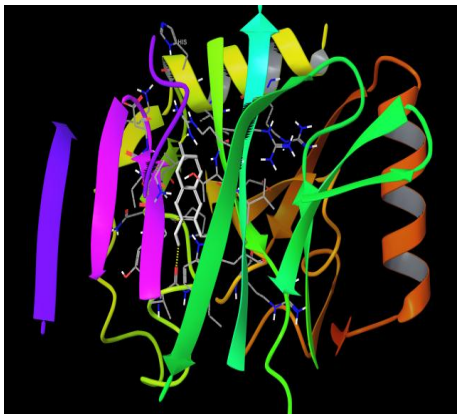
Esculetin



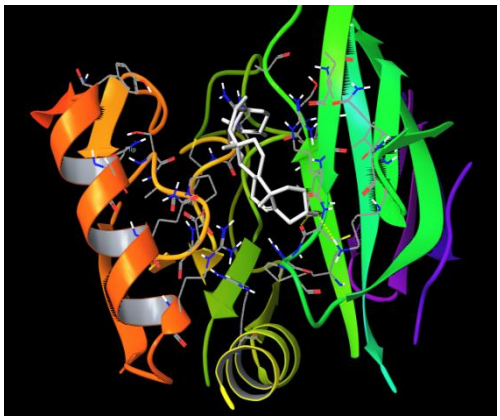
Esculin



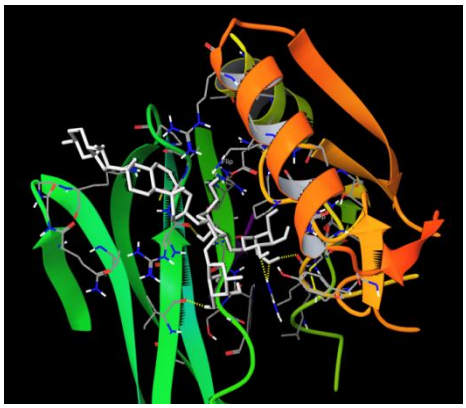
Methyl caffeate



Scopletin

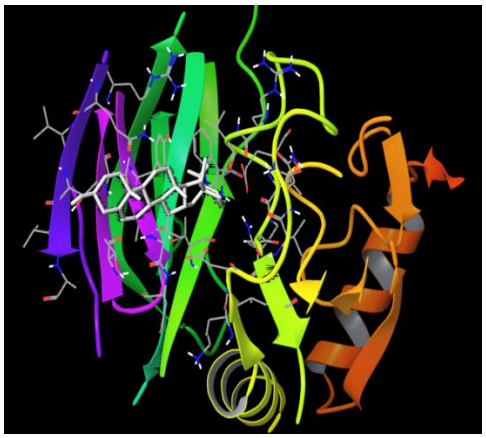


Solanidine

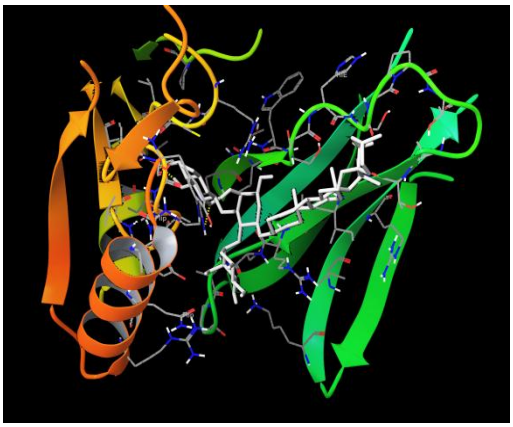


Solanine

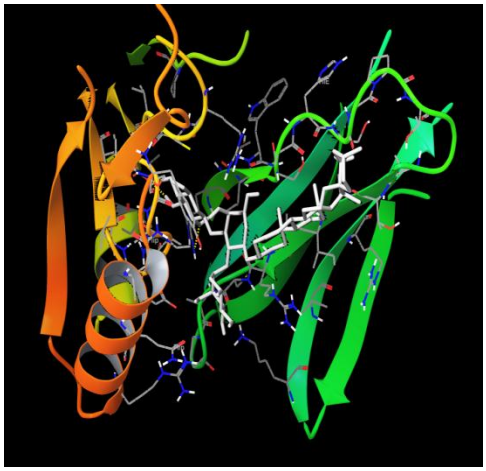
Figure 10 (Part-III): Compounds docked against 3GCX



Solasodine



Solasonine



Tomatidenol

Atorvastatin

It is considered that lesser the G score value greater is the binding of the ligand with the protein. From the G score values, it is observed that Solanine, Esculin, Solasonine, Esculetin, α-Solamargine, Apigenin, Scopletin and Caffeic acid showed a G score value ranging between -11.32 to -5.3 indicating a good score. It is also seen that Methyl caffeate, Coumarin, Solanidine, Diosgenin and Solasodine had a G score value ranging between -4.6 to – 0.88 3 indicating a moderate score as compared to Atorvastatin.

C. Drug likeness

All the 15 molecules were subjected to *in silico* evaluation using **Molinspiration Cheminformatics Software** to evaluate drug likeness. It can be accessed online for calculation of important molecular properties such as partition co-efficient, binding with human serum albumin, percentage human oral absorption, number of hydrogen bond donors and acceptors for the most important drug targets like GPCR ligands, kinase inhibitors, ion channel modulators and nuclear receptors. Drug likeness parameters are tabulated in **Table 12.**

Table 12: Prediction of drug likeness

S.No	Molecule	QPlogP_o/W	QPlogkhsa	% oral human absorption	Rule of five
1	Carpesterol	8.44	2.305	100	2
2	Solasodine	5.83	1.304	100	1
3	Apigenin	1.92	0.05	75.23	0
4	Caffeic acid	0.558	-0.798	54.28	0
5	Coumarin	1.39	-0.554	94.30	0
6	Diosgenin	6.124	1.642	100	1
7	Esculetin	0.118	-0.593	69.66	0
8	Esculin	-1.596	-1.012	39.72	0
9	Methyl caffeate	1.008	-0.407	75.97	0
10	Scopletin	0.968	-0.492	85.73	0
11	Solanidine	5.118	1.435	95.89	1
12	α-Solamargine	0.473	-0.943	9.92	3
13	Solanine	-0.791	-1.045	0	3
14	Solasonine	-1.14	-1.046	0	3
15	Tomatidenol	5.325	1.143	100	1
16	Atorvastatin	6.812	1.124	70.53	2

From the table,

- $QPlogP_o/W$ - It is used to predict the Partition co-efficient of the molecules. The values are normally in the range between -2.0 to 6.5.
- QPlogkhsa - It is used to predict the binding with human serum albumin. The values are normally ranges between -1.5 to 1.5.
- Percent human oral absorption - It is used to predict the human oral absorption on 0 to 100% scale. Absorption values normally ranges between 25-80%.
- Rule of 5 - Lipinski's rule said that molecules should possess MW<500, donor HB≤5, accept HB≤10, $QPlogP_o/W$<5. Molecules that statisfy this rule are considered drug-like.

Results indicates that

- All the molecules possess good partition co-efficient except Carpesterol.
- Carpesterol and Diosgenin does not show desirable binding with human serum albumin whereas all other molecules have good effective binding with human serum albumin.
- Absorption value less than 25% are, α-Solamargine, Solanine, Solasonine and more than 80% are Carpesterol, Diosgenin, Solasodine, Coumarin, Scopletin, Solanidine.
- Apigenin, Caffeic acid, Esculetin, Esculin, Methyl caffeate, Scopletin, Coumarin have the value as 0; Solasodine, Diosgenin, Solanidine, Tomotidenol have the value as 1; Carpesterol have the value as 2; α-Solamargine, Solanine, Solasonine have the value as 3.

Among 15 compounds, Carpesterol, Solasodine, Apigenin, Caffeic acid, Coumarin, Diosgenin, Esculetin, Esculin, Methyl caffeate, Scopletin, Solanidine were seen to possess drug likeness.

Based on the *in silico* studies the molecules Diosgenin, Esculin, Methyl caffeate, Scopletin, Solanidine, Solasonine were found to be safe, orally effective and possess good G scores.

SUMMARY

Herbal medicines are found to be effective in the treatment of various aliments but the major lacuna is lack of proper scientific validation. Hence the present study is aimed at investigating the selected plant *Solanum virginianum* Linn., for the hyperlipidemia.

The plant *Solanum virginianum* Linn., belong to Family Solanaceae, is claimed to be useful for reducing the fats but the claim has not been scientifically validated.

Authentication of the plant material plays a key role in herbal medicine. The fruits of *Solanum virginianum* Linn., were collected from the waste lands in Krishnagiri district, Tamilnadu in the month of August, 2015 and authenticated by Prof. Sasikala Ethirajulu, Botanist, Siddha Central Research Institute, Arumbakkam, Chennai-600106.

Antihyperlipidemic activity of the ethanolic extract of fruits of *Solanum virginianum* Linn., using the Cholesterol diet induced model of hyperlipidemia. The parameters evaluated were body weight changes and serum lipid profile. Administration of standard (Atorvastatin 2mg/kg b.w), ethanolic extract of fruits of *Solanum virginianum* Linn., at 200mg/kg and 400mg/kg significantly ($P<0.001$) reduced the body weight and normalized the serum lipid profile. This confirms the antihyperlipidemic activity of fruits of *Solanum virginianum* Linn.,

In silico studies like toxicity, docking and drug likeness were performed for establishing safety and identifying the mechanism of action of some of the selected molecules which have already been isolated from *Solanum virginianum* Linn.,

Toxicity screening was done *in silico* using **OSIRIS** property explorer. Solanine, Esculin, Solasonine, Diosgenin, α-Solamargine, Solanidine, Tomatidenol, Methyl caffeate, Scopletin, Carpesterol, Solasodine were found to be non- toxic.

In docking studies Solanine, Esculin, Solasonine, Esculetin, α-Solamargine, Apigenin, Scopletin and Caffeic acid were good G score. Methyl caffeate, Coumarin, Solanidine, Diosgenin, Solasodine were posses moderate G score with the use of **Glide**.

Carpesterol, Solasodine, Apigenin, Caffeic acid, Coumarin, Diosgenin, Esculetin, Esculin, Methyl caffeate, Scopletin, Solanidine and Tomatidenol were posses more drug likeness with the use of **Molinspiration Cheminformatics** Software.

Based on the *in silico* studies the molecules Diosgenin, Esculin, Methyl caffeate, Scopletin, Solanidine, Solasonine were found to be safe, orally effective with proven activity in terms of G score.

CONCLUSION

It is thus concluded that ethanolic extract of fruits of *Solanum virginianum Linn.,* have significant antihyperlipidemic activity. The presence of phytochemicals such as alkaloids, flavonoids, coumarins, phenolic compounds and glycosides are responsible for antihyperlipidemic activity.

It is also concluded from *in silico* studies of already isolated compounds of *Solanum virginianum* Linn., against 3GCX confirming the activity through its G score. Most of the molecules are safe and orally effective. So the molecules can be docked with other target proteins on overall prospective on the mechanism of action of these isolated compounds.

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